Supporting Information

The Effect of Copper Salts on the Peptide Amide Bond Formation using Peptide Thioesters

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Experimental Section

General Methods.

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. All solvents were peptide synthesis grade. Protected amino acids, 2-(1H-benzotriazole-1-yl)-1, 1, 3, 3-tetramethyluronium hexafluorophosphate (HBTU), N-hydroxybenzotriazole (HOBt), 3-carboxypropansulfonamide, 2-Chlorotrityl resin (loading: 0.8-1.6 mmol/g) were purchased from CalBiochem-NovaBiochem GmbH. The Champion I resin (loading: 0.34 mmol/g) was purchased from Biosearch Technologies Inc. (Novato, CA, USA). Diisopropylcarbodiimide, (diethylamino)sulfur trifluoride (DAST), 2,4,6-trimethylpyridine (TMP), 3-Ethylmercaptopropionate, Sodium thiophenate 2-(methylsulfonyl)ethoxycarbonyl succinimid (Msc-OSu), N-t-butoxycarbonyloxysuccinimide (Boc-OSu) and diisopropylethylamine (DIEA) were purchased from Fluka (Buchs, Switzerland). N-hydroxybenzotriazole Copper salt [$Cu(OBt)_2$] was purchased from Luxemburg Industries (PAMOL) LTD, Israel. Peptide synthesis was performed on a Pioneer Synthesizer (Applied Biosystems). Analytical HPLC was performed on a Merck-Hitachi chromatograph equipped with a UV-detector L-7400 (wavelength monitoring, 220nm), a L-7100 pump and Phenomenex (Jupiter) C-18 columns (250 x 4.6 mm, 5 μm, 100Å; 250 x 4.6 mm, 5 μm, 300Å) at a flow rate of 1 mL/min. Preparative HPLC was performed on a Dionex chromatograph equipped with a Diode Array UVD1700S, a pump P580 and a Waters Delta-PakTM C-18 column (100 x 250 mm, 15 μm); the operating flow rate was 20 ml/ min. The solvent system was always: eluent A, water (0.1% TFA); eluent B, MeCN (0.095% TFA).The LC-MS was performed on a Finnigan Mat LCQ, combined with a Hewlett Packard HPLC Series 1100, equipped with a Vydac C-18 column (250 x 4.6 mm, 5 μm). The eluting solvent system was: eluent A, water (0.1% TFA); eluent B, MeCN (0.1% TFA).

Synthesis of C-erminal peptide fragments N1, N2, Z38[17-38] and Z38[18-38]. The peptides were assembled by machine-assisted Fmoc/t-Bu chemistry using 1g of Rink-amide-ChampionsI resin (0.33 mmol/g) for N1 and N2, and using 0.5g of 2-Chlorotityl resin (0.45 mmol/g) for Z38[17-38] and Z38[18-38]. Side-chain protections were as follows: Fmoc-

Arg(2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl), Fmoc-Asp(O-t-butyl), Fmoc-Gln(trityl), FmocGlu(O-t-butyl), Fmoc-His(trityl), Fmoc-Lys(t-butoxycarbonyl), Fmoc-Lys(ivDde), Fmoc-Ser(t-butyl), Fmoc-Thr(t-butyl), and Fmoc-Tyr(t-butyl). The Synthesis performed on Pioneer Synthesizer (Applied Biosystems), 5-fold excess of acylating agent was used over the resin amino groups, with the following coupling times : N1: residues Ala5-Phe1, 60 min.; N2: residues Ala5-Phe1, 60 min.; Z38[17-38] and: residues Leu19-Asp38 double coupling of 45 min.; for residues Lys32 and Lys34 ivDde N-ε- protection was used; Z38[18-38] : residues Ala18-Asp38 double coupling of 45 min.; for residues Lys32 and Lys34 ivDde N-ε- protection was used.

After the last piperidine cycle, the resin was washed with DMF, MeOH and CH2Cl2 and dried to constant weight in vacuo. *Removal of N-terminal and side-chain protecting groups*. For the peptide N1 this procedure was done only to check the quality of the peptide on the resin. For N2 and half of Z38[17-38] and Z38[18-38] resin-peptide, in a typical experiment, 500 mg of peptide-resin were treated with 15 mL of TFA 88%, phenol 5%, triisopropylsilane 2%, water 5% (Reagent B) for 2 h at 25ºC. The resin was filtered and rinsed with TFA. The TFA solution was added dropwise to screw cap centrifuge tubes containing MTBE with a TFA/MTBE ratio of 1/10; after centrifugation at 3200 x g (30 min), the ether was removed and the peptide precipitate was dissolved in 50% MeCN/water and lyophilised.

Peptide N1. The crude material contained approximately 94% of target material as judged by analytical HPLC (Phemomenex (Jupiter) C-18 column, 250 x 4.6 mm, 5 μm) gradient 5-80%B over 30 min, t_R of the target peptide 9.8 min.

Peptide N2. The crude material was 170 mg, (yield, 94%). It contained approximately 92% of the target material as judged by analytical HPLC (Phemomenex (Jupiter) C-18 column, 250 x 4.6 mm, 5 μm) gradient 5-80%B over 30 min., t_R of the target peptide 10.1 min. The peptide was purified on a Waters Delta-PakTM C-18 column. In a typical run, the peptide (20 mg) was dissolved in 5 ml MeCN/H2O/TFA (5:94.9:0.1 v/v) and immediately loaded onto the preparative column at a flow rate of 3 ml/min. The flow was raised to 20 ml/min and the peptide eluted with a linear gradient between 10-30%B over 30 min. The fractions containing the desired peptide (>98% purity) were pooled and lyophilized: yield, 16 mg, 80%. Mass spectrometry analysis: calculated (average isotopic composition) 542.6 daltons, found 542.5.

Z38[17-38]. The crude material was 520 mg, (yield, 77%). It contained approximately 67% of the target material as judged by analytical HPLC (Phemomenex (Jupiter) C-18 column, 250 x 4.6 mm, 5 μm, 300Å) gradient 5-80%B over 30 min, t_R of the target peptide 16.1 min. The peptide was purified on a Waters Delta-PakTM C-18 column. In a typical run, the peptide (50

mg) was dissolved in 5 ml MeCN/H2O/TFA (20:79.9:0.1 v/v) and immediately loaded onto the preparative column at a flow rate of 3 ml/min. The flow was raised to 20 ml/min and the peptide eluted with a linear gradient between 20-20%B over 5 min, 20-45%B over 40 min. The fractions containing the desired peptide (>98% purity) were pooled and lyophilized: yield, 20 mg, 60%. Mass spectrometry analysis: calculated (average isotopic composition) 2997.45 daltons, found 2997.9.

Z38[18-38]. The procedure used and the yield obtained were identical to the previous peptide.

Synthesis of N-terminal peptide thioester fragments T1, T2, T3, Z38[1-16] and Z38[1-17]. For the preparation of peptide thioesters we followed the procedure already reported[1,2] The protecting groups, synthetic protocols and work-up were the same as reported for peptides N1-N3 (see above) except that the N-terminal residues were capped with 4 eq of 2-(methylsulfonyl)ethoxycarbonyl succinimid (Msc-Osu). Coupling times were: T1: all residues, 60 min.; T2: all residues, 60 min.; T3: all residues, 60 min.; Z38[1-16]: all residues, 45 min. and double coupling; Z38[1-17]: all residues, 45 min. and double coupling.

Peptide T1. The crude material was 260 mg, (yield, 90%). It contained approximately 88% of the target material as judged by analytical HPLC (Phemomenex (Jupiter) C-18 column, 250 x 4.6 mm, 5 μm, 100Å) gradient 5-80%B over 30 min, t_R of the target peptide 17.6 min. The peptide was purified on a Waters Delta-PakTM C-18 column. In a typical run, the peptide (20 mg) was dissolved in 5 ml MeCN/H2O/TFA (5:94.9:0.1 v/v) and immediately loaded onto the preparative column at a flow rate of 3 ml/min. The flow was raised to 20 ml/min and the peptide eluted with a linear gradient between 15-35%B over 30 min. The fractions containing the desired peptide (>98% purity) were pooled and lyophilized: yield, 11.5 mg, 65%. Mass spectrometry analysis: calculated (average isotopic composition) 845.01 daltons, found 845.1.

Peptide T2. The crude material was 250 mg, (yield, 86%) containing 88% of the target material. The peptide was purified on a Waters Delta-PakTM C-18 column. In a typical run, the peptide (20 mg) was dissolved in 5 ml MeCN/H2O/TFA (5:94.9:0.1 v/v) and immediately loaded onto the preparative column at a flow rate of 3 ml/min. The flow was raised to 20 ml/min and the peptide eluted with a linear gradient between 15-35%B over 30 min. The fractions containing the desired peptide (>98% purity) were pooled and lyophilized: yield, 11.1 mg, 63%. Ion-spray mass spectrometry: calculated. Mass spectrometry analysis: calculated (average isotopic composition) 859.04 daltons, found 859.1.

Peptide T3. The crude material was 277 mg, (yield, 85%) containing 89% of the target material. The peptide was purified on a Waters Delta-PakTM C-18 column. In a typical run,

the peptide (20 mg) was dissolved in 5 ml MeCN/H2O/TFA (5:94.9:0.1 v/v) and immediately loaded onto the preparative column at a flow rate of 3 ml/min. The flow was raised to 20 ml/min and the peptide eluted with a linear gradient between 15-35%B over 30 min. The fractions containing the desired peptide (>98% purity) were pooled and lyophilized: yield, 11 mg, 62.5%. Mass spectrometry analysis: calculated (average isotopic composition) 901.14 daltons, found 901.2.

Z38[1-16]. The crude material was 385 mg, (yield, 85%). It contained approximately 75% of the target material as judged by analytical HPLC (Phemomenex (Jupiter) C-18 column, 250 x 4.6 mm, 5 μm, 300Å) gradient 5-80%B over 30 min, t_R of the target peptide 16.1 min. The peptide was purified on a Waters Delta-PakTM C-18 column. In a typical run, the peptide (30 mg) was dissolved in 5 ml MeCN/H2O/TFA (20:79.9:0.1 v/v) and immediately loaded onto the preparative column at a flow rate of 3 ml/min. The flow was raised to 20 ml/min and the peptide eluted with a linear gradient between 20-20%B over 5 min, 20-45%B over 40 min. The fractions containing the desired peptide (>98% purity) were pooled and lyophilized: yield, 22.5 mg, 75% yield. Mass spectrometry analysis: calculated (average isotopic composition) 2119.45 daltons, found 2120.4.

Z38[1-17]. The procedure used and the yield obtained were identical to the previous peptide.

Introduction of Boc and Msc groups. Msc protection of the N^{α}-amino group of last amino acid for T1, T2, T3, Z38[1-16] and Z38[1-17] peptides was performed immediately after the coupling of last the amino acid and the last cycle of Fmoc-deprotection. The reaction was performed overnight using 4 equiv. of Msc-OSu under agitation in DMF. The introduction of Boc protection was performed only for the purified peptide Z38 [1-16] and Z38[1-17]. In a typical experiment, to a DMF solution of 50 mg (23.5 μmol, 10 mM) of purified peptide thioester 15 mg (70μmol, 3 equiv.) of N-t-butoxycarbonyloxysuccinimide (Boc-OSu) and 6.5 μL (47μmol, 2 equiv.) Collidine were added and left under agitation overnight at room temperature. Then the mixture is precipitated in cold MTBE and washed three times. Peptide dried, solubilized in water/acetonitrile and lyophilized.

1) Ingenito R., Bianchi E., Fattori D. and Pessi A.: **Solid Phase Synthesis of Peptide C-Terminal Thioesters by Fmoc/t-Bu Chemistry.** *J. Am. Chem. Soc.*, **1999**, *121*, 11369-11374.
2) Ingenito R., Dreznjak D., Guffler S. and Wenschuh H.: **Efficient Loading of Sulfonamide Safety-Catch linkers by Fmoc Amino Acid Fluorides.** *Org. Lett.*, **2002**, *4*, 1187-1188.

Amide bond formation by means of thioesters/CuX (X=Cl, Br or I)/TMP on Solid Support. The purified peptide thioester T1 (11.1 mg, 13.2 μmol) or T2 (11.4 mg, 13.2 μmol) or T3 (11.9 mg, 13.2 μmol) was weighted in an 0.5-ml polypropylene eppendorff vial and 67 μl of DMF were added (0.2 M final concentration) and then 10 mg resin-peptide N1 (0.265 mmol/g) previously swelled in DMF for 20 min. was added into the reaction vial. To this mixture CuX (X=Cl, 2.7 mg, 26.5 μmol; X=Br, 3.8 mg, 26.5 μmol; X=I, 5 mg, 26.5 μmol;) and then 3.6μl (10 eq) of Collidine were added. The solution was vortexed and centrifugated to let the resin to stand completely immerged in the acylation solution. The reaction was left on the termomixer at 45°C for 16h under agitation. *Workup and deprotection of side-chain protecting groups*. The solution was filtered off using a 2 ml polypropylene syringe equipped with a bottom 20 μm polyethylene filter and the resin was washed 3 x 1 ml DMSO, 3 x 1 ml DCM, 3 x 1 ml DMF, 3 x 1 ml DCM, 2 x 1 ml ether and dried under vacuum. The drained resin was treated with 1 ml of 88%TFA, 2% triisopropylsilane, 5% water for 2 h at 21°C. The TFA solution was added dropwise to screw cap centrifuge tubes containing MTBE with a TFA/MTBE ratio of 1/10; after centrifugation at 3200 x g (30 min), the ether was removed and the peptide precipitate was dissolved in 50% MeCN/water and lyophilized. Mass spectrometry analysis: T1N1 calculated (average isotopic composition) 1294.5 daltons, found 1294.6; T2N1 calculated (average isotopic composition) 1308.5 daltons, found 1308.6; T3N1 calculated (average isotopic composition) 1350.6 daltons, found 1350.8.

Amide bond formation by means of thioesters/Cu(OBt)$_2$/TMP on Solid Support. The purified peptide thioester T1 (11.1 mg, 13.2 μmol) or T2 (11.4 mg, 13.2 μmol) or T3 (11.9 mg, 13.2 μmol)was weighted in an 0.5-ml polypropylene eppendorff vial together with Cu(OBt)$_2$ (8.7 mg, 26.5 μmol) and then 10 mg peptide-resin N1 (0.265 mmol/g) previously swelled in DMF for 20 min. was added into the reaction vial. As solvent, 67 μl of DMF were added (0.2 M final concentration) and 3.6μl (10 eq) of Collidine were added. The solution was vortexed and centrifugated to let the resin to be totally wet and immerged in the solution itself. The reaction was left on the termomixer at 45°C for 16h under agitation. *Workup and deprotection of side-chain protecting groups*. The solution was filtered off using a 2 ml polypropylene syringe equipped with a bottom 20 μm polyethylene filter and the resin was washed 3 x 1 ml DMSO, 3 x 1 ml DCM, 3 x 1 ml DMF, 3 x 1 ml DCM, 2 x 1 ml ether and dried under vacuum. The drained resin was treated with 1 ml of 88% TFA, 2%

triisopropylsilane,5% water for 2 h at 21°C. The TFA solution was added dropwise to screw cap centrifuge tubes containing MTBE with a TFA/MTBE ratio of 1/10; after centrifugation at 3200 x g (30 min), the ether was removed and the peptide precipitate was dissolved in 50% MeCN/water and lyophilized. Mass spectrometry analysis: T1N1 calculated (average isotopic composition) 1294.5 daltons, found 1294.6; T2N1 calculated (average isotopic composition) 1308.5 daltons, found 1308.6; T3N1 calculated (average isotopic composition) 1350.6 daltons, found 1350.8.

Amide bond formation by means of thioesters/CuX (X=Cl, Br or I)/Thiophenate on Solid Support. The purified peptide thioester T1 (11.1 mg, 13.2 μmol) or T2 (11.4 mg, 13.2 μmol) or T3 (11.9 mg, 13.2 μmol) was weighted in an 0.5-ml polypropylene eppendorff vial and 67 μl of DMF (0.2 M final concentration) were added. To the solution of peptide thioester Sodium thiophenate (1mg,~3 eq) was added and left for 30 min., then 10 mg peptide-resin N1 (0.265 mmol/g) previously swelled in DMF for 20 min. was added into the reaction vial together with CuX (X=Cl, 2.7 mg, 26.5 μmol; X=Br, 3.8 mg, 26.5 μmol; X=I, 5 mg, 26.5 μmol). The solution was vortexed and centrifugated to let the resin to stand completely immerged in the solution itself. The reaction was left on the termomixer at 45°C for 16h under agitation. *Workup and deprotection of side-chain protecting groups*. The solution was filtered off using a 2 ml polypropylene syringe equipped with a bottom 20 μm polyethylene filter and the resin was washed 3 x 1 ml DMSO, 3 x 1 ml DCM, 3 x 1 ml DMF, 3 x 1 ml DCM, 2 x 1 ml ether and dried under vacuum. The drained resin was treated with 1 ml of 88% TFA, 2% triisopropylsilane, 5% water for 2 h at 21°C. The TFA solution was added dropwise to screw cap centrifuge tubes containing MTBE with a TFA/MTBE ratio of 1/10; after centrifugation at 3200 x g (30 min), the ether was removed and the peptide precipitate was dissolved in 50% MeCN/water and lyophilized. Mass spectrometry analysis: T1N1 calculated (average isotopic composition) 1294.5 daltons, found 1294.6; T2N1 calculated (average isotopic composition) 1308.5 daltons, found 1308.6; T3N1 calculated (average isotopic composition) 1350.6 daltons, found 1350.8.

Amide bond formation by means of thioesters/$Cu(OBt)_2$/TMP in solution phase. The purified peptide thioester T1 (9.3 mg, 11 μmol) or T3 (10 mg, 11 μmol) was weighted in an 2-ml polypropylene eppendorff vial together with the purified peptide N2 (2 mg, 3.6 μmol) and $Cu(OBt)_2$ (6 mg, 18 μmol). 736 μl of DMF were added (5 mM final concentration) as solvent

and 2.5 µl (5 equiv.) of Collidine were added as base. The reaction was left on the termomixer at 45°C under agitation and was checked by sampling the solution from the reaction vial as follows: 1.5 µl mother solution was diluted in 48.5 µl of MeCN/H2O/TFA (49.9:50:0.1 v/v) and 20 µl immediately loaded onto the analytical HPLC column, Phemomenex-Jupiter C-18 column (250 x 4.6 mm, 5 µm, 100Å), gradient 5-80%B over 30 min, t_R of the T1N2 target peptide 16.1 min; t_R of the T3N2 target peptide 18.2 min. *Workup*. The solution was rotary evaporated in a round-bottom flask of 10 ml and the peptides triturated with cold ether two times and then precipitated and separated from the ether phase, dissolved in MeCN/water and lyophilized. T1N2 calculated (average isotopic composition) 1253.4 daltons, found 1253.6; T3N2 calculated (average isotopic composition) 1309.6 daltons, found 1309.8.

Amide bond formation by means of thioesters/CuCl in solution phase. The purified peptide thioester T1 (9.3 mg, 11 µmol) or T3 (10 mg, 11 µmol) was weighted in an 2-ml polypropylene eppendorff vial, dissolved in 736 µl of DMF (5 mM final concentration) and Sodium thiophenate (0.5 mg, 3.6 µmol) was added. The mixture was left on the termomixer at 45°C under agitation for 30 min and then the purified peptide N2 (2 mg, 3.6 µmol) was added. The reaction was left on the termomixer at 45°C under agitation and was checked by sampling the solution form the reaction vial as follows: 1.5 µl mother solution was diluted in 48.5 µl of MeCN/H2O/TFA (49.9:50:0.1 v/v) and20 µ immediately loaded onto the analytical HPLC column, Phemomenex-Jupiter C-18 column (250 x 4.6 mm, 5 µm, 100Å), gradient 5-80%B over 30 min, t_R of the T1N2 target peptide 16.1 min; t_R of the T3N2 target peptide 18.2 min. *Workup*. The solution was rotary evaporated in a round-bottom flask of 10 ml and the peptides triturated with cold ether two times and then precipitated and separated from the ether phase, dissolved in MeCN/water and lyophilized. T1N2 calculated (average isotopic composition) 1253.4 daltons, found 1253.6; T3N2 calculated (average isotopic composition) 1309.6 daltons, found 1309.8.

LCQ and HPLC analysis

The unpurified materials coming from ligation reactions, immediately after the cleavage and workup, were subjected to HPLC and LCQ analysis to determine the yield and the extent of loss of configuration at ligation site chosen.

To determine the yield of ligation in all cases, either on solid support and in solution, the HPLC conditions used were : 5-80(%B) in 30 min., 80-95(%B) in 1 min., 95-95 (%B) in 5 min., 95-5 (%B) in 2 min.

To determine the extent of loss of configuration at ligation site chosen, for the ligation on solid support (T1N1, T2N1, T3N1) the HPLC conditions used were : 30-32 (%B) in 30 min., 32-95 (%B) in 3 min., 95-95 (%B) in 5 min., 95-30 (%B) in 1 min.

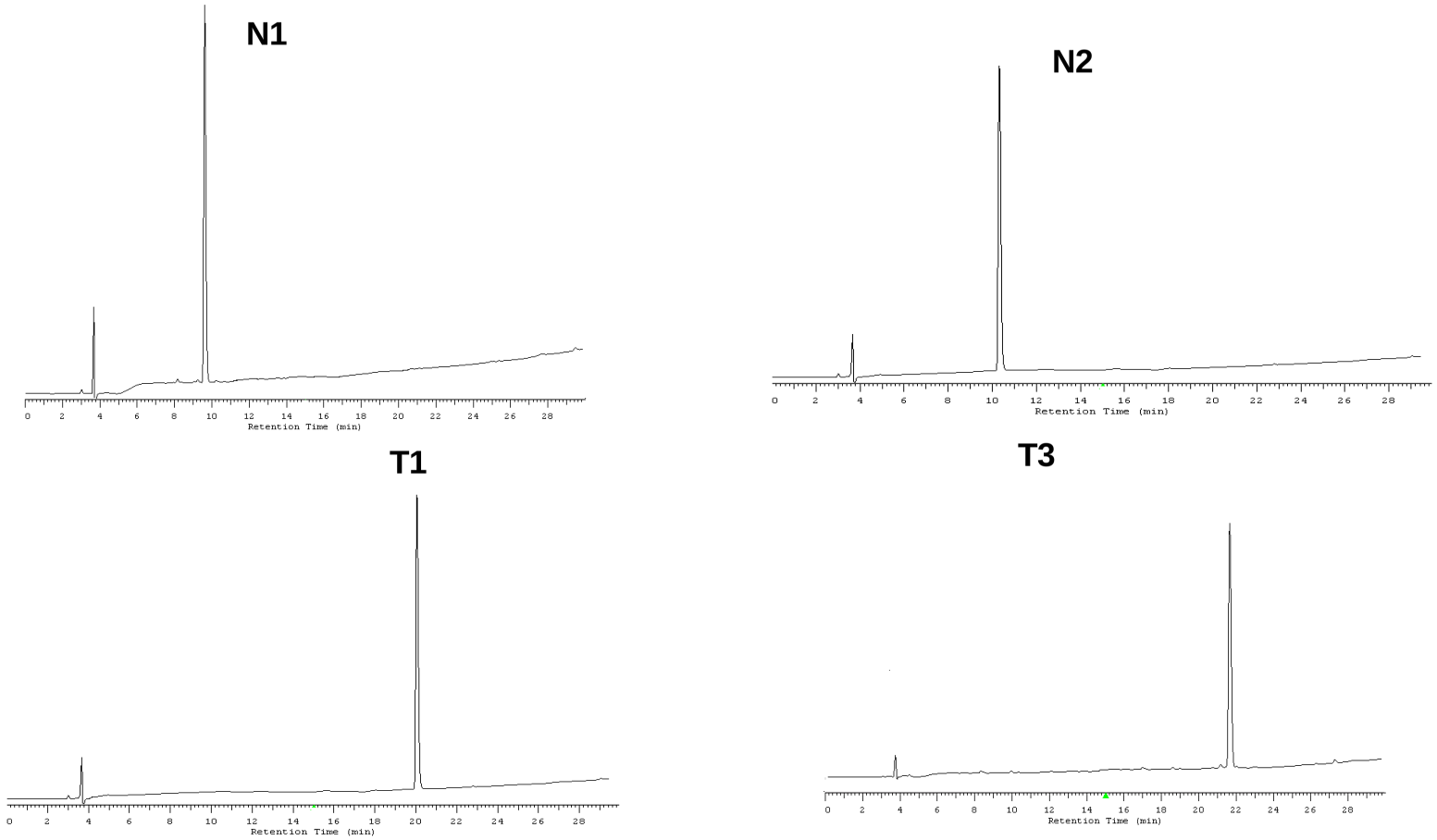
For the ligation in solution phase (T1N2, T2N2, T3N2) the HPLC conditions used were : 27-32 (%B) in 30 min., 32-95 (%B) in 3 min., 95-95 (%B) in 5 min., 95-27 (%B) in 1 min.

The column used was a and Phenomenex (Jupiter) C-18 columns (250 x 4.6 mm, 5 μm, 100Å).

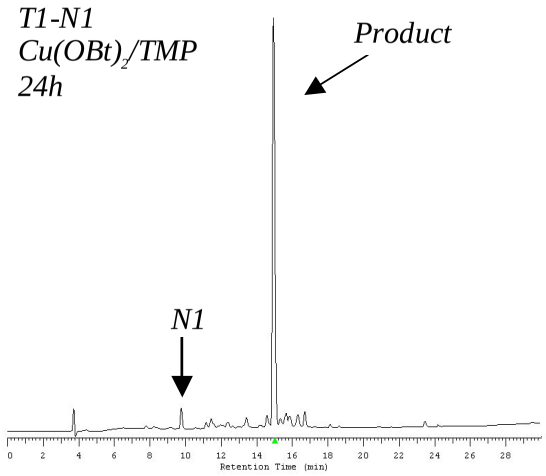
Segment Condensation of Z38 by means of thioesters/Cu(OBt)$_2$/TMP in solution phase. 18.5 mg (8.3 μmol) of purified peptide thioester **Z38[1-16**] and 5 mg (1.6 μmol) of purified peptide **Z38[17-38]** were weighted in an 2-ml polypropylene eppendorff vial. To the reaction vial, 333 μL of DMF (5 mM final concentration) and 2.6 mg (8.3 μmol) Cu(OBt)$_2$ were added together with 1.2 μL (8.3 μmol) of Collidine. The reaction was left on the termomixer at 45°C under agitation and was checked by sampling the solution form the reaction vial as follows: 1.5 μl mother solution was diluted in 20 μl of MeCN/H2O/TFA (49.9:50:0.1 v/v) and immediately loaded 10 μL onto the analytical HPLC column, Phemomenex-Jupiter C-18 column (250 x 4.6 mm, 5 μm, 300Å), gradient 5-50%B over 30 min. and 5 μL analysed by LC/MS. *Workup*. The solution was rotary evaporated in a round-bottom flask of 10 ml and the peptides triturated with cold ether two times. 200 μL of neat TFA was added to the tritured material to remove Boc protection from the peptides. Then the mixture was precipitated again in cold MBTE and peptides washed again, dried, solubilized in 50% MeCN/water and lyophilized. *Removal of ivDde.* A mixture of 3% Hydrazine in DMF (200μL) was added to the lyophilised material for 10 min. and peptide precipitated in cold MBTE, washed three times, dried, solubilized in 50% MeCN/water and lyophilised. The crude material was purified by RP-HPLC semipreparative Phenomenex (Jupiter) C-18 column (250 x 10 mm, 5 μm, 300Å). In a typical run, 10 mg of crude peptide was dissolved in 2 ml MeCN/H2O/TFA (10:89.9:0.1 v/v) and immediately loaded onto the preparative column at a flow rate of 2 ml/min. The flow was raised to 8 ml/min and the peptide eluted with a linear gradient between 10-10%B over 5 min, 10-40%B over 40 min. The fractions containing the desired peptide

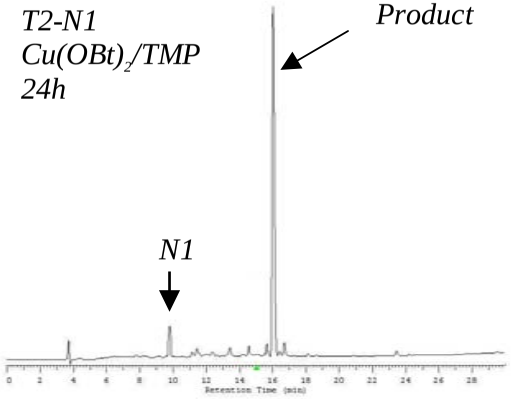
(>95 % purity) were pooled and lyophilized: 2 mg, 20 % overall yield. Mass spectrometry analysis: calculated (average isotopic composition) 4562.06 daltons, found 4562.6.

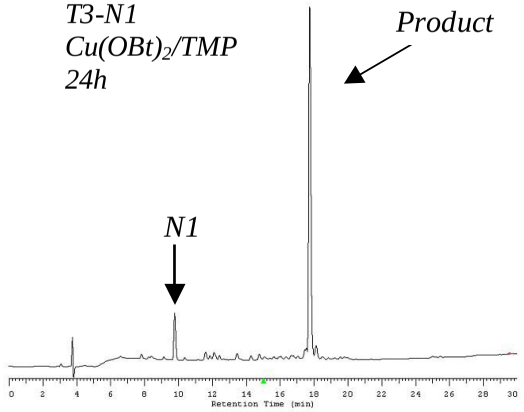
HPLC profiles of the purified peptides N1 (FYGKA), N2 (FYGSA), T1 (Msc-LYRAGCOSR), T3 (Msc-LYRALCOSR) used for the Copper-mediated ligation .

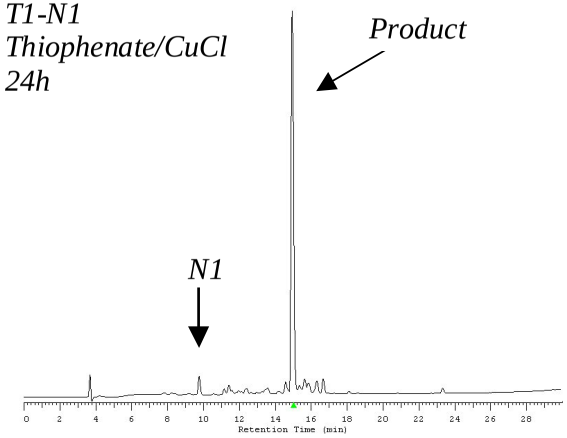


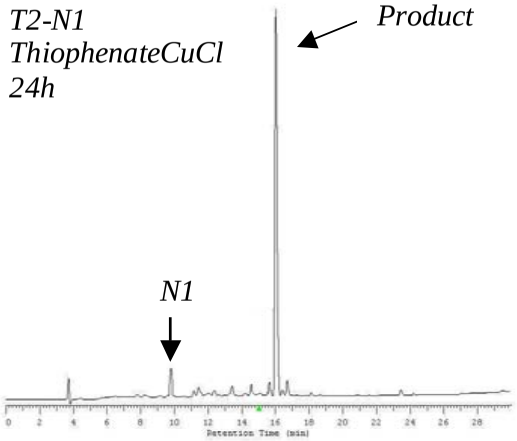
HPLC Profiles of crude peptides resulting from ligation on solid phase in the presence of different additives at junction points Gly-Phe, Ala-Phe and Leu_Phe (24h reaction time). See Table 1 and 2 in the manuscript.

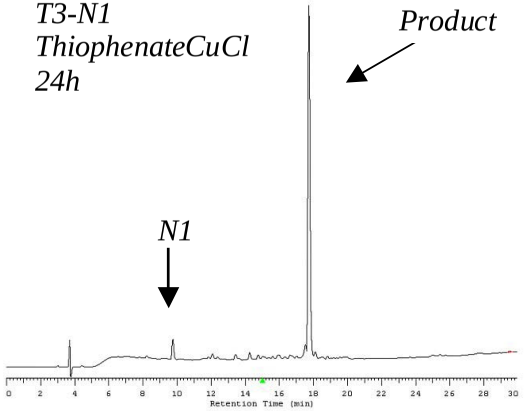








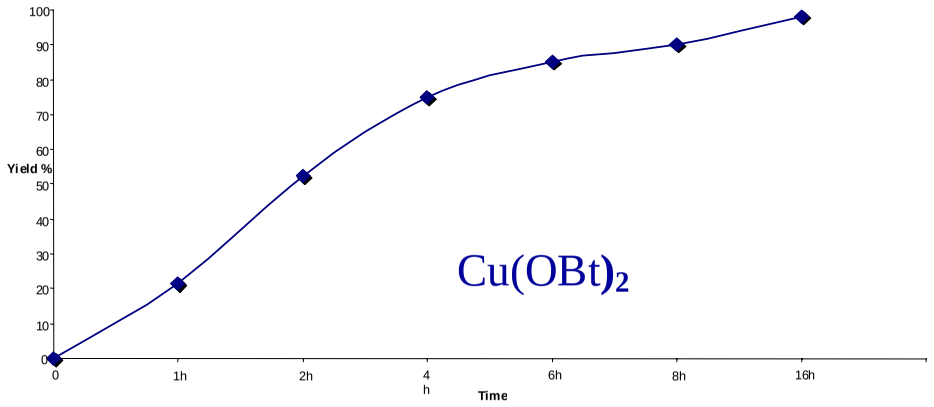




Time course for reaction of peptide thioesters with resin bound peptide N1: (Panel A) T1 in the presence of Cu(OBt)2/TMP; (Panel B): T1 in the presence of CuCl/thiophenate,

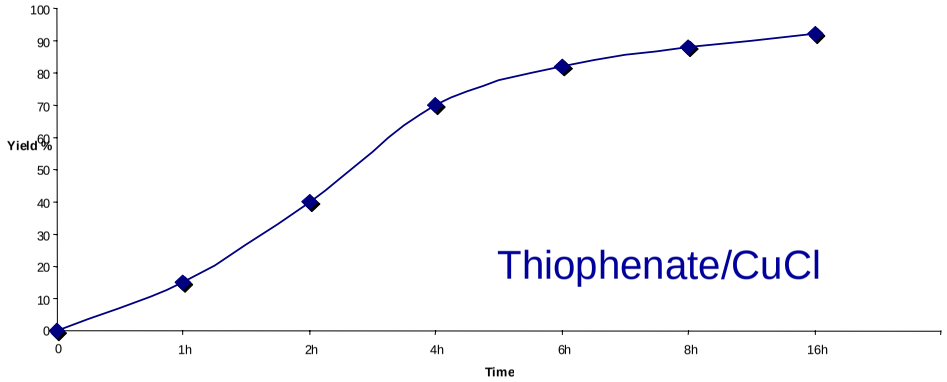
A

Time (h)	Acylation yield (%)
0	0
1	21
2	52
4	75
6	84
8	90
16	90

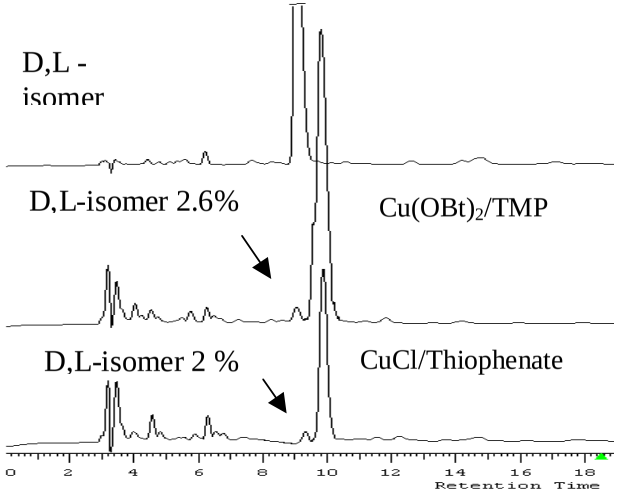


B

Time (h)	Acylation yield (%)
0	0
1	15
2	40
4	70
6	82
8	88
16	90



Superimposition of HPLC profiles showing the loss of configuration obtained using different methods for reaction of peptide thioesters T3 with resin bound peptide N1 in the presence of Cu(OBt)2/TMP and in the presence of CuCl/thiophenate, compared with the D,L-standard (D-Leu at the C-terminal). See Table 2 in the manuscript.

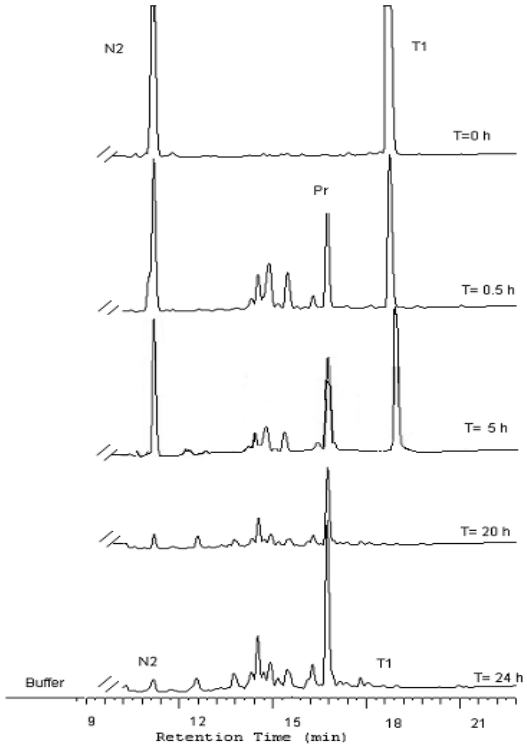


Time course for the solution phase reaction of T1 with N2 under the addition of $Cu(OBt)_2$/TMP.Gradient 5-80 (%B).
Rt of product 17.1 min.

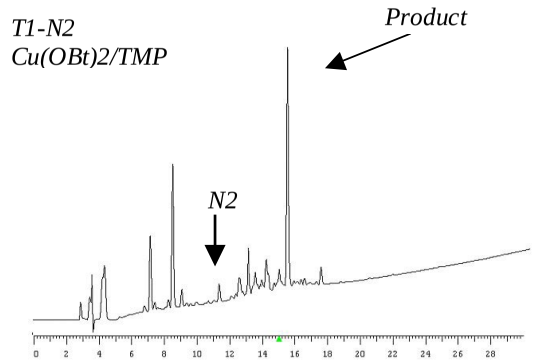
(T1) Msc-LYRAG$_{COSR}$ NH_2FYGSA$_{CONH_2}$ (N2)

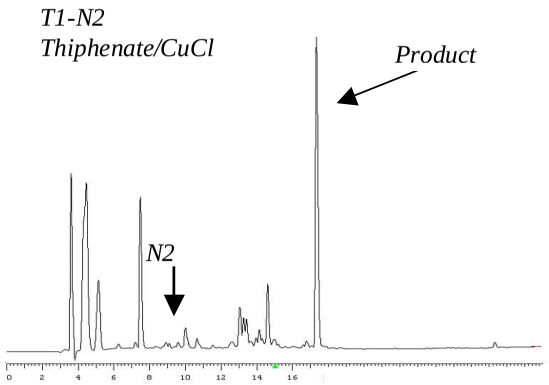
↓

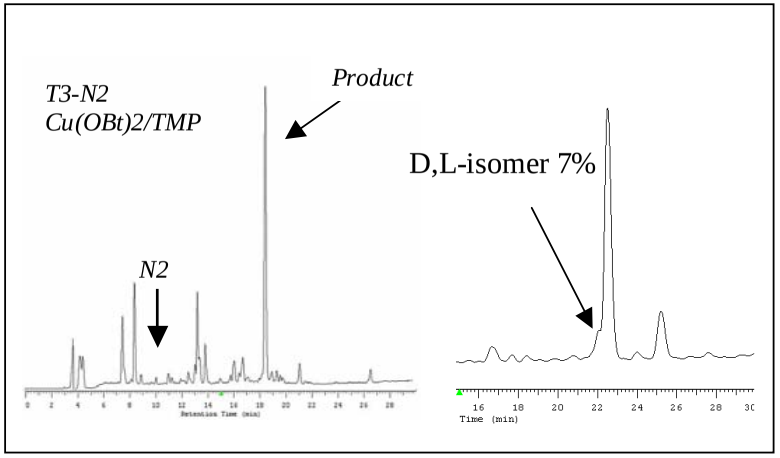
Msc-LYRAG$_{CONH}$FYGSA$_{CONH_2}$

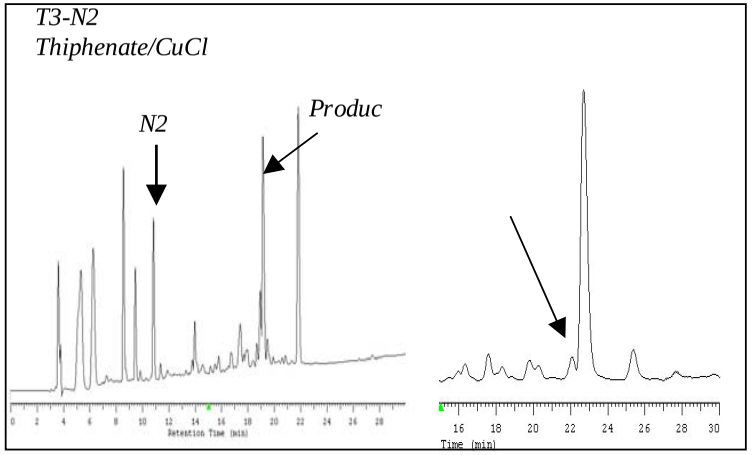


HPLC Profiles of crude peptides, after workup as described above, resulting from ligation in solution phase in the presence of different additives at junction points Gly-Phe and Leu-Phe (after 24 h reaction time) with the corresponding D,L isomesr See ***Table 3*** *in the manuscript.*





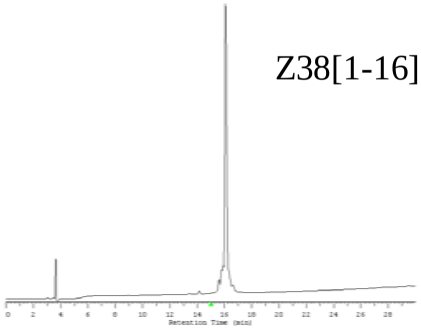




Synthesis of Z38 mini domain of Protein A:copper-mediated ligation at site Glu-Ala by using $Cu(OBt)_2$/TMP as additives in solution. HPLC profiles of purified fragments Z38[1-16]COSR *and* Z38[17-38].

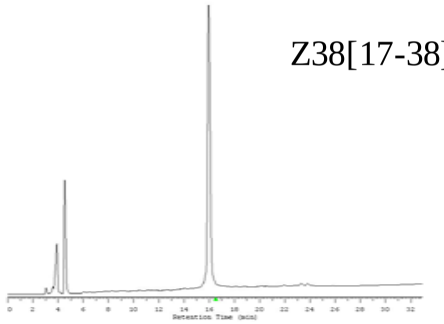
NH_2AVAQSFNMQQQRRFYE**COSR**

Z38[1-16]COSR



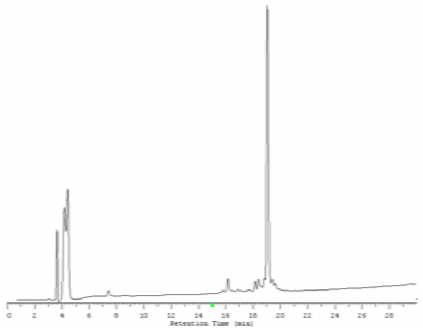
NH_2ALHDPNLNEEQRNAKIKSIRDDCOOH

Z38[17-38]

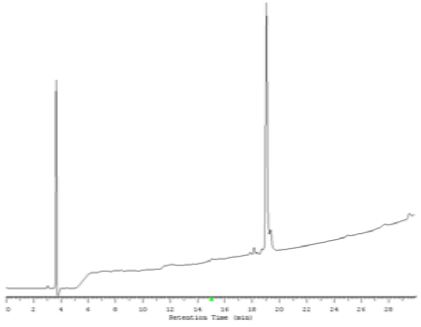


HPLC profiles of protected fragments Z38[1-16]COSR *and* Z38[17-38] *for the copper–mediated ligation at junction site Glu-Ala.*

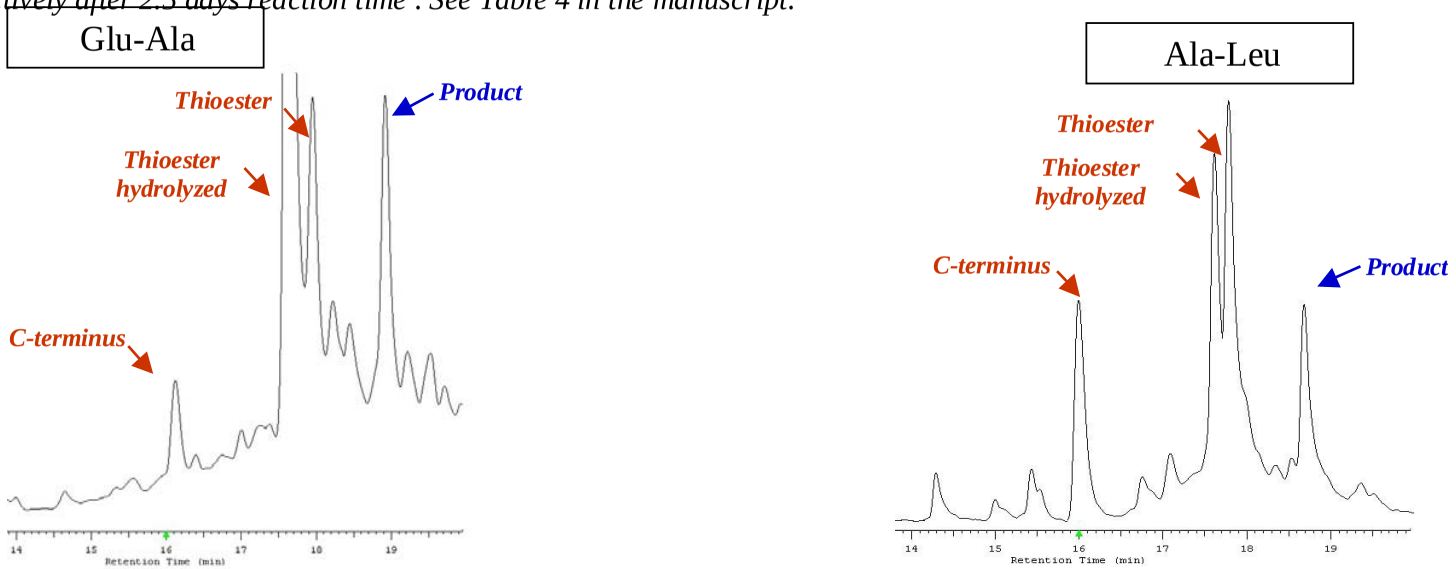
Boc-NH-AVAQSFNMQQQRRFYE**COSR**



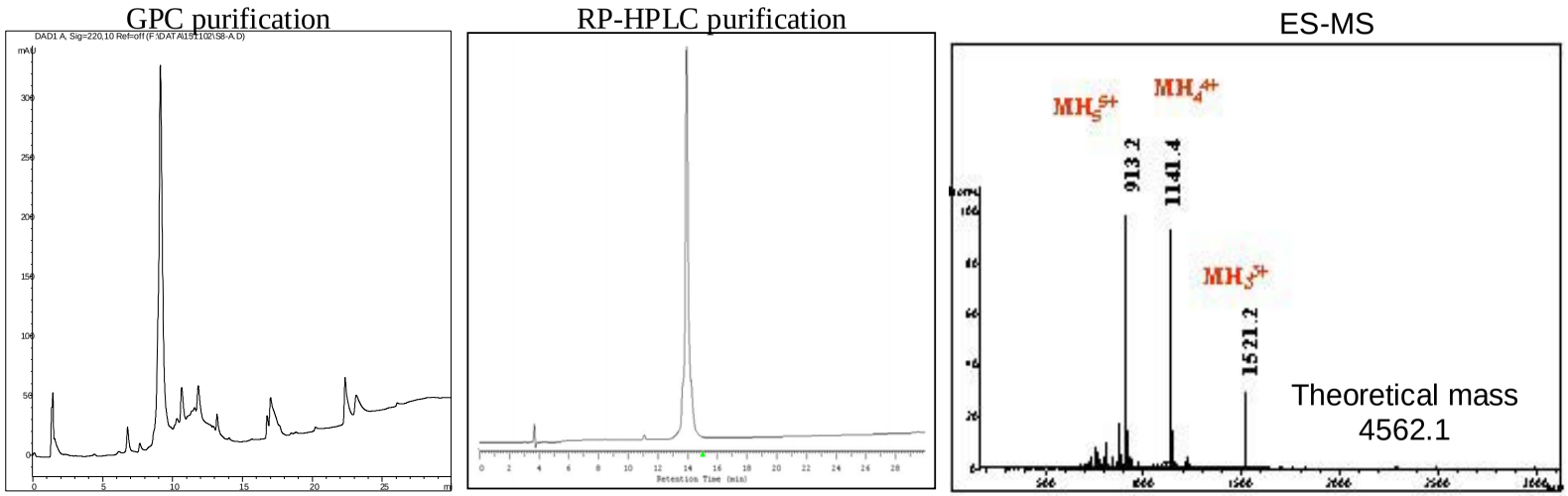
NH_2ALHDPNLNEEQRNAK$_{DDe}$IK$_{Dde}$SIRDDCOOH



HPLC profiles of the copper mediated ligation in sokution when Cu(OBt)2/TMP were used as additives for the ligation sites Glu-Ala and Ala-Leu respectively after 2.5 days reaction time . See Table 4 in the manuscript.



GPC purification, HPLCprofile of purified material and Ms analysis of ligated matrial after workup and deprotection.



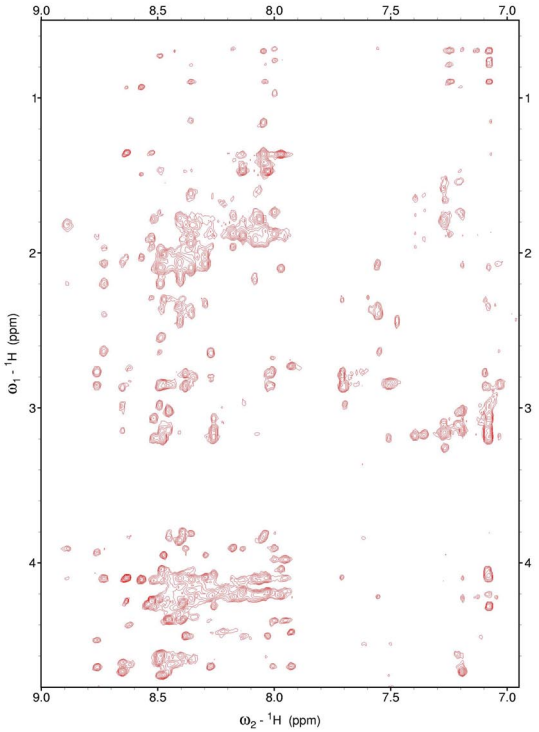
NMR analysis of Z38 protein: *Comparison between 2D NOESY Spectra of wt Protein Z38 and Cu-ligated*
2D ^{1}H-^{1}H NOESY by 600 MHz (150 ms Mischzeit, 8°C, 50 mM Na-Acetat-Buffer pH 5.1, 8% D2O) form 0.75 mM ^{Cu}Z38 (red) and 1.5 mM ^{wt}Z38 (black) with 48 and. 24 scans.

Illustrations : Comparison of the 2D NOESY spectra of ^{Cu}Z38 und ^{Fest}Z38

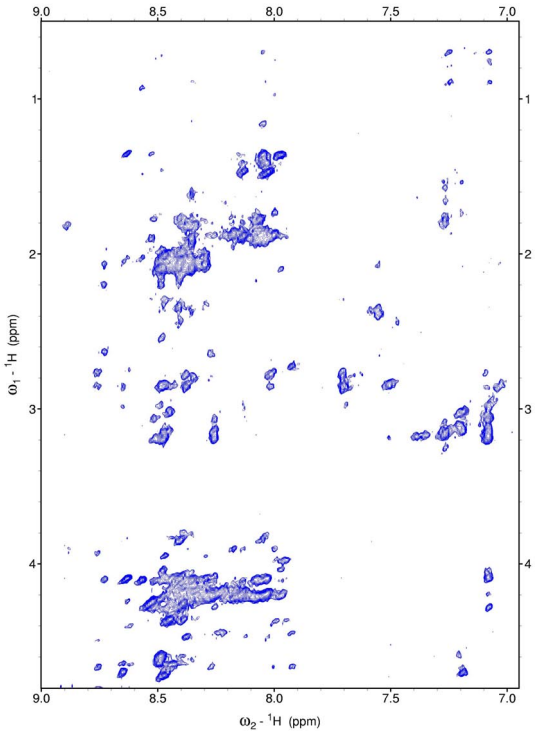
(A) *2D 1H-1H NOESY at 600MHz Finger print regions comparison*

The clearly quite overlapping Finger Print Regions of both ^{Cu}Z38 and ^{wt}Z38 proteins shown the same 3D folding in water solution.

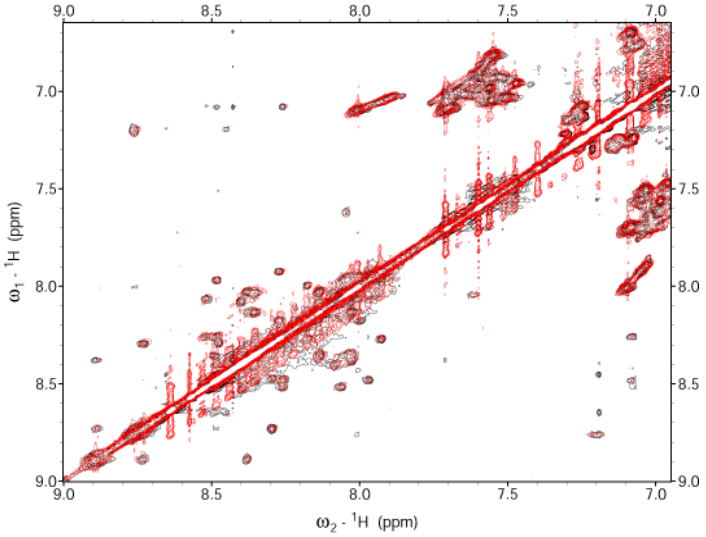
wt



Cu-ligated



(B) Similar to (A) the signals from ^{Cu}Z38 und ^{wt}Z38 in the HN-HN-Region fits very good.

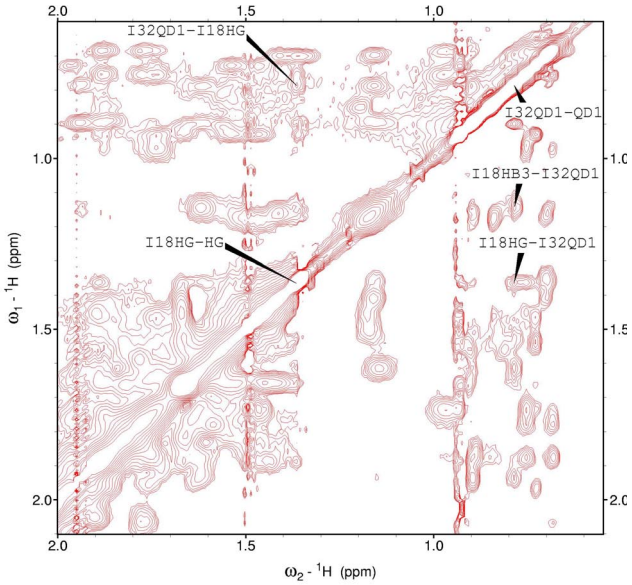


(C) The *Long-range NOE cross-peaks comparison between Ile18 and Ile32*

In the picture C is reported a Long-range NOE cross-peaks comparison between Ile18 and Ile32 of the protein Z38, both wild-type and copper synthesized. The results found for the 3D folding of ^{Cu}Z38 and ^{wt}Z38 based on this comparison were in agreement with known 3D structure published already [1]. These two amino acids either in ^{Cu}Z38 and ^{wt}Z38 were found at distance about of 6Å, which is in the range of expressed protein, and showing that both proteins could assume the same folding reported for the crystal structure of B-domain of protein A in the active binding form [1]. As published before in the 3D structure, where these two amino acids were found in a range distance of circa 4Å [2].

(1) Deisenhofer, J. (1981) Biochemistry, 20, 2361-2370.
(2) Starovasnik, M.A., Braisted, A.C. & Wells, J.A. (1997) Proc. Natl. Acad. Sci. USA, 94, 10080 – 10085.

wt



Cu-ligated